Infrared Spectroscopy

1. Introduction

Why Should I Study This?

Spectroscopy is the study of the interaction of energy and matter. Absorption of energy of different magnitudes causes different changes in matter. The magnitude of energy absorbed is determined by the structure of the matter under study. If we understand how the structure of matter influences the energy absorbed, we can work backwards to elucidate certain features of molecular structure such as the presence of functional groups (IR spectroscopy), arrangement of hydrogen and carbon atoms (NMR spectroscopy) or the extent of conjugation (UV/visible spectroscopy).

Determination of the complete molecular structure of an unknown substance is made easier when we have more clues. One useful clue is the molecular formula, which is provided by mass spectrometry. However, the formula alone is usually not enough for us to propose a single structure, because even a small collection of atoms often has several constitutional isomers. For example, a molecule with the formula C2H6O might be methyl ether (CH3OCH3) or ethyl alcohol (CH3CH2OH). More complex formulas can lead to dozens or even hundreds of possibilities. What additional spectroscopic clues might be useful to differentiate between these constitutional isomers?

The names of these molecules suggest a solution: one is an ether and the other an alcohol, so a spectroscopic method that determines the *functional*

groups of a molecule will differentiate between these two constitutional isomers of C2H6O.

A method that achieves this goal is **infrared (IR) spectroscopy**. In this technique, we expose the molecule in question to infrared photons. As we will learn in the next few sections, functional groups absorb infrared photons of characteristic energies. We then make a plot of photon energy versus intensity of absorption, called the **infrared spectrum**. *Therefore IR spectroscopy allows us to deduce the functional groups that are present and absent in a molecule* (1).

IR spectroscopy is a powerful tool for the determination of molecular structure, and thus it is of fundamental importance for the modern organic chemist (research scientist and student alike).

1.1 Short history of the technique:

Infrared radiation was discovered by Sir William Herschel in 1800 [2]. Herschel was investigating the energy levels associated with the wavelengths of light in the visible spectrum. Sunlight was directed through a prism and showed the well known visible spectrum of the *rainbow colors*, i.e, the visible spectrum from blue to red with the analogous wavelengths or frequencies [3,4] (see Fig.1).

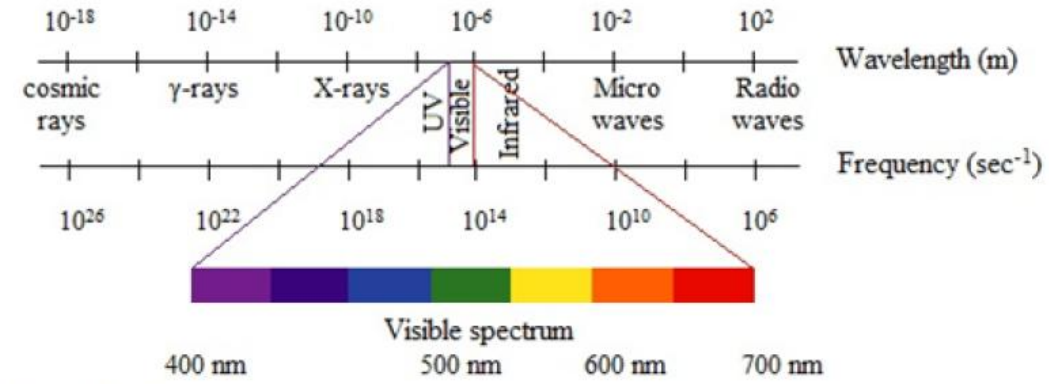


Fig. 1. The electromagnetic spectrum.

Spectroscopy is the study of interaction of electromagnetic waves (EM) with matter. The wavelengths of the colors correspond to the energy

levels of the rainbow colors. Herschel by slowly moving the thermometer through the visible spectrum from the blue color to the red and measuring the temperatures through the spectrum, he noticed that the temperature increased from blue to red part of the spectrum. Herschel then decided to measure the temperature just below the red portion thinking that the increase of temperature would stop outside the visible spectrum, but to his surprise he found that the temperature was even higher. He called these rays, which were below the red rays "non colorific rays" or invisible rays, which were called later "infrared rays" or IR light. This light is not visible to human eye. A typical human eye will respond to wavelengths from 390 to 750 nm. The IR spectrum starts at 0.75 nm. One nanometer (nm) is 10-9 m The Infrared spectrum is divided into, Near Infrared (NIRS), Mid Infrared (MIRS) and Far Infrared (FIRS) [5-6].

1.2 Infrared Absorptions

For a molecule to show infrared absorptions it must possess a specific feature, i.e. an electric dipole moment of the molecule must change during the vibration. This is the *selection rule* for infrared spectroscopy. Figure 2 illustrates an example of an 'infrared-active' molecule, a *heteronuclear* diatomic molecule. The dipole moment of such a molecule changes as the bond expands and contracts. By comparison, an example of an 'infrared-inactive' molecule is a *homonuclear* diatomic molecule because its dipole moment remains zero no matter how long the bond. An understanding of molecular symmetry and group theory is important when initially assigning infrared bands.

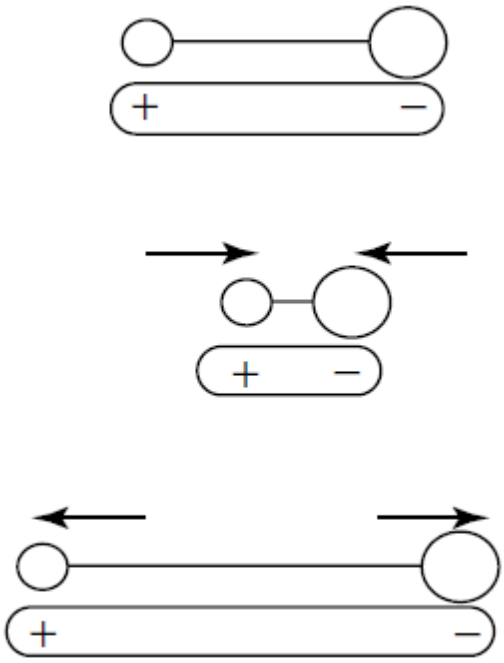


Figure 2 Change in the dipole moment of a heteronuclear diatomic molecule.

Infrared absorptions are not infinitely narrow and there are several factors that contribute to the broadening. For gases, the Doppler effect, in which radiation is shifted in frequency when the radiation source is moving towards or away from the observer, is a factor. There is also the broadening of bands due to the collisions between molecules. Another source of line broadening is the finite lifetime of the states involved in the transition. From quantum mechanics, when the Schr¨odinger equation is solved for a system which is changing with time, the energy states of the system do not have precisely defined energies and this leads to lifetime broadening. There is a relationship between the lifetime of an excited state and the bandwidth of the absorption band associated with the transition to the excited state, and this is a consequence of the *Heisenberg Uncertainty Principle*. This relationship demonstrates that the shorter the lifetime of a state, then the less well defined is its energy.

1.3 Normal Modes of Vibration

The interactions of infrared radiation with matter may be understood in terms of changes in molecular dipoles associated with vibrations and rotations. In order to begin with a basic model, a molecule can be looked upon as a system of masses joined by bonds with spring-like properties. Taking first the simple case of diatomic molecules, such molecules have three degrees of translational freedom and two degrees of rotational freedom. The atoms in the molecules can also move relative to one other, that is, bond lengths can vary or one atom can move out of its present plane. This is a description of stretching and bending movements that are collectively referred to as *vibrations*. For a diatomic molecule, only one vibration that corresponds to the stretching and compression of the bond is possible. This accounts for one degree of vibrational freedom.

Vibrations can involve either a change in bond length (*stretching*) or bond angle (*bending*) (Figure 3). Some bonds can stretch in-phase (*symmetrical* stretching) or out-of-phase (*asymmetric* stretching), as shown in Figure 4. If a molecule has different terminal atoms such as HCN, ClCN or ONCl, then the two stretching modes are no longer symmetric and asymmetric vibrations of similar bonds, but will have varying proportions of the stretching motion of each group. In other words, the amount of *coupling* will vary.

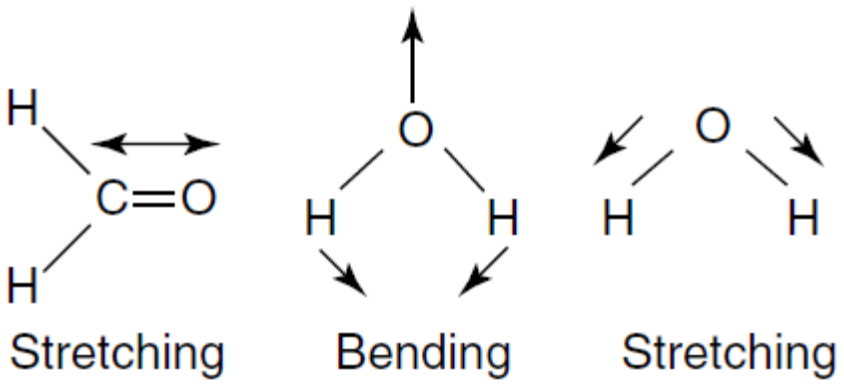


Figure 3 Stretching and bending vibrations.

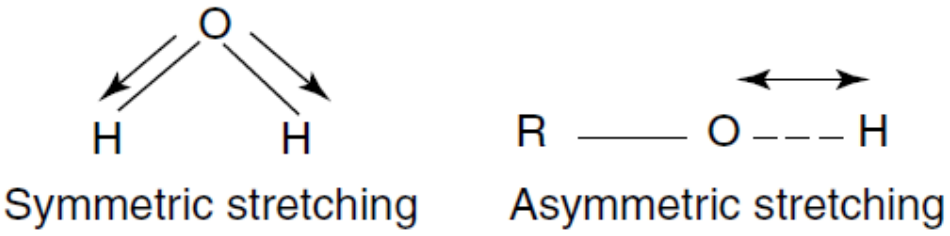


Figure 4 Symmetric and asymmetric stretching vibrations.

Bending vibrations also contribute to infrared spectra and these are summarized in Figure 5. It is best to consider the molecule being cut by a plane through the hydrogen atoms and the carbon atom. The hydrogens can move in the same direction or in opposite directions in this plane, here the plane of the page. For more complex molecules, the analysis becomes simpler since hydrogen atoms may be considered in isolation because they are usually attached to more massive, and therefore, more rigid parts of the molecule. This results in *in-plane* and *out-of-plane* bending vibrations, as illustrated in Figure 6.

As already mentioned, for a vibration to give rise to the absorption of infrared radiation, it must cause a change in the dipole moment of the molecule. The larger this change, then the more intense will be the absorption band. Because of the difference in electronegativity between carbon and oxygen, the carbonyl group is permanently polarized, as shown in Figure 7. Stretching this bond will increase the dipole moment and, hence, C=O stretching is an intense absorption. In CO2, two different stretching vibrations are possible: (a) symmetric and (b) asymmetric (Figure 8). In practice, this ʻblack and whiteʼ situation does not prevail. The change in dipole may be very small and, hence, lead to a very weak absorption.

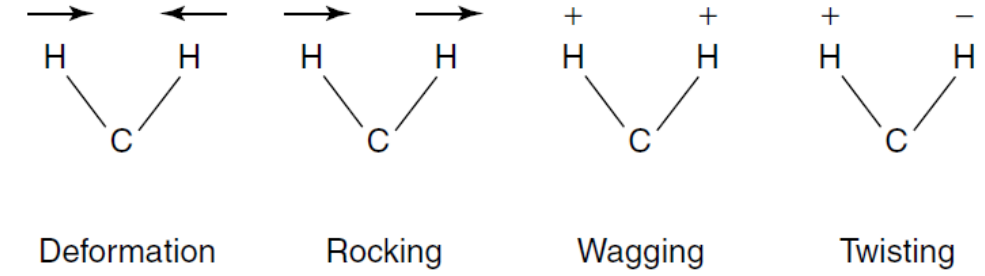


Figure 5 Different types of bending vibrations.

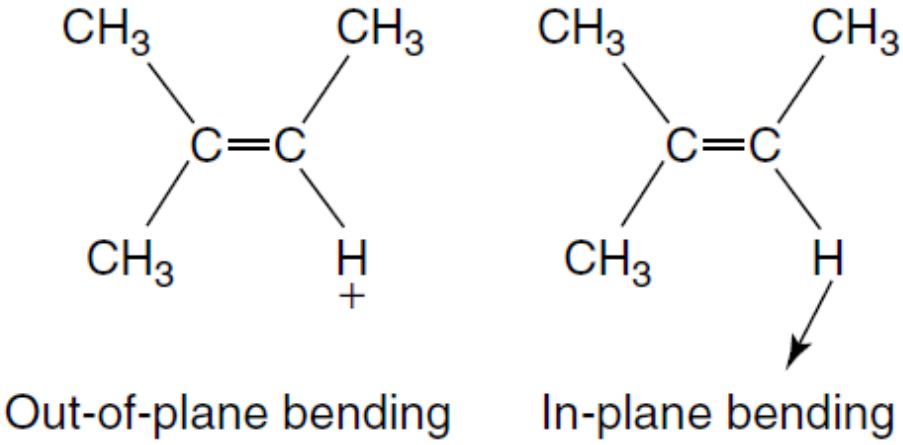


Figure 6 Out-of-plane and in-plane bending vibrations.

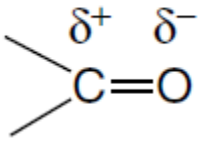


Figure 7 Dipole moment in a carbonyl group.

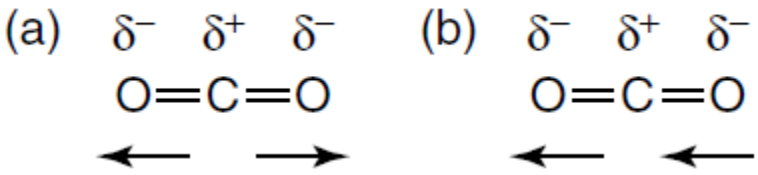


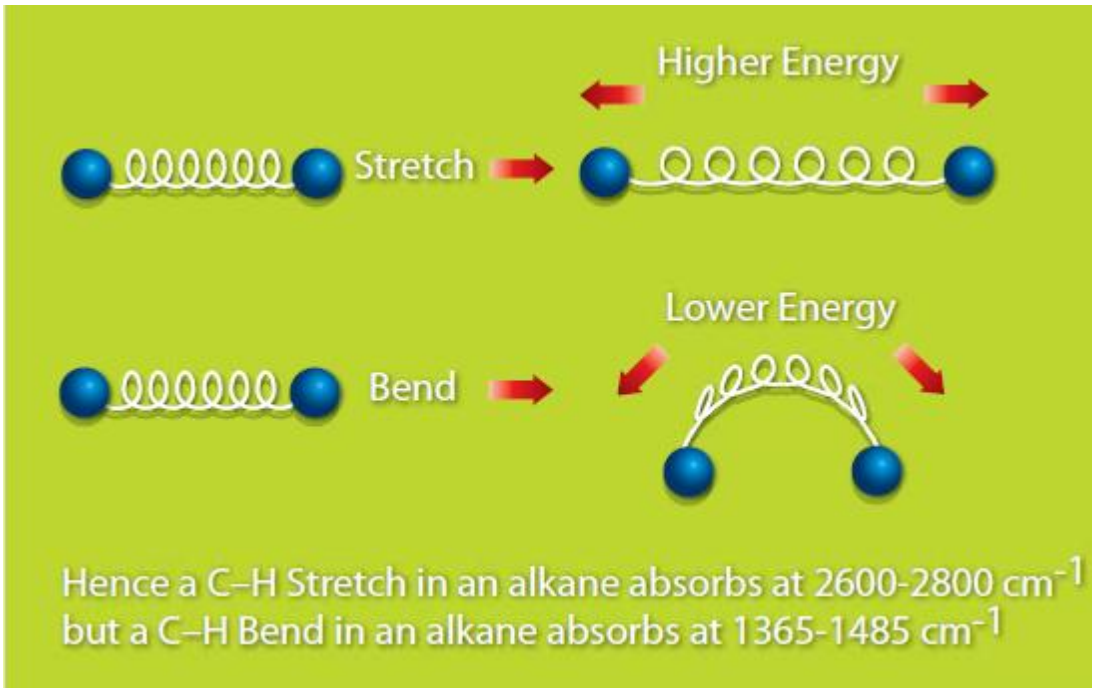
Figure 8 Stretching vibrations of carbon dioxide.

1.4 Factors that affect vibrations

4.1-Type of Vibration

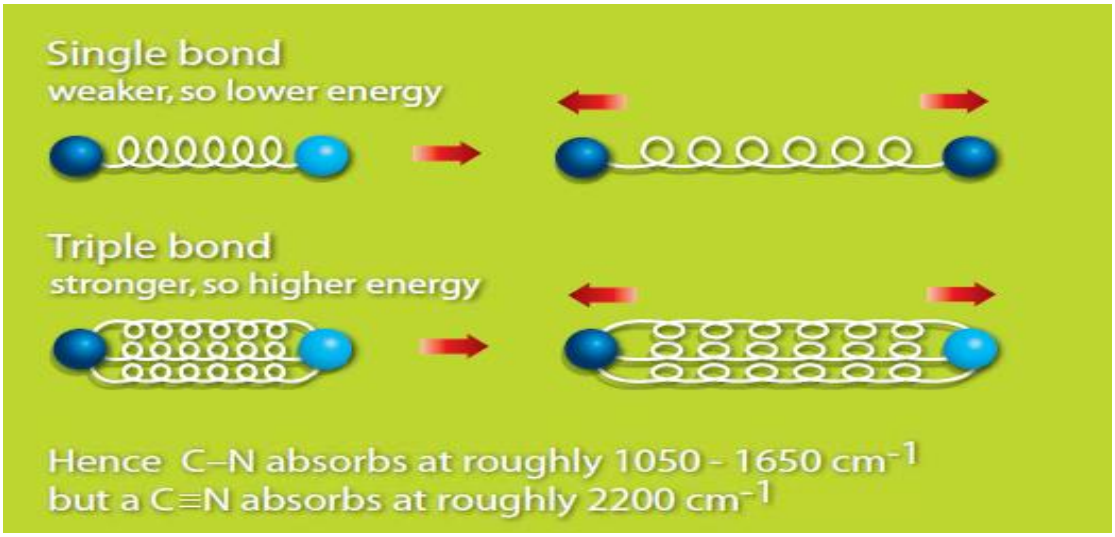
The energy absorbed when particular bonds vibrate depends on several factors. To get your head around this it is helpful to use an analogy; you can think of a bond as a spring between two atoms. Imagine trying

to bend or stretch the spring. Generally it is easier to bend than stretch, so bending vibrations are of lower energy than stretching vibrations for the same bond. Therefore, absorptions due to bending tend to occur at lower wavenumbers than stretches fig.9.



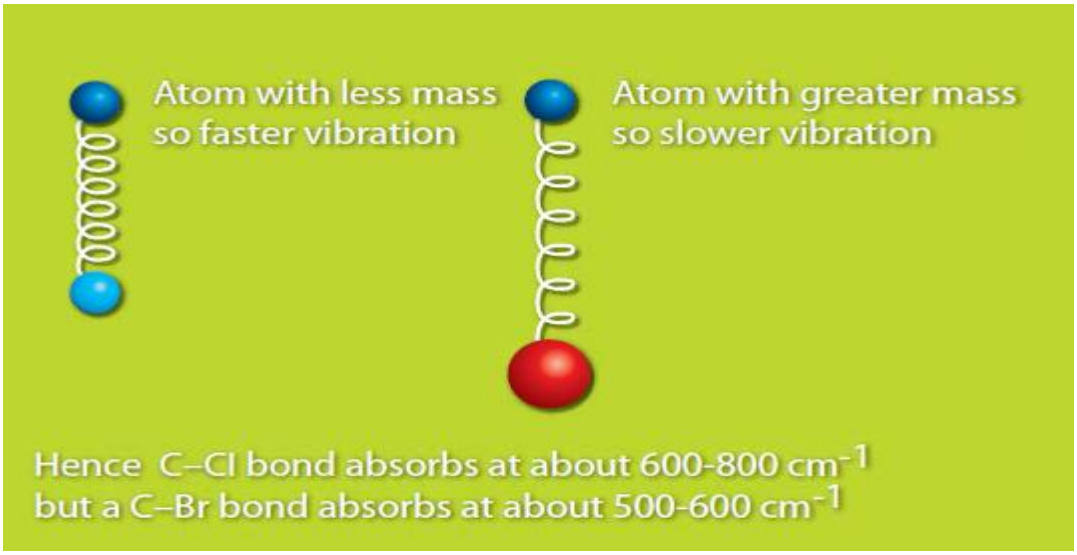
4.2-Strength of Bonds

You can think of a strong bond as a stiff spring. This will need more energy to make the ‘spring’ bond vibrate, so stronger bonds absorb at higher wavenumbers fig.10.



4.3-Mass of Atoms

Finally the atoms in the bond can be thought of as masses at the end of the spring. Heavy masses on a spring vibrate more slowly than lighter ones. Using this analogy we can imagine that heavier atoms vibrate at a lower frequency than lighter ones. Therefore you would expect a C-Br bond to absorb at a lower frequency than a C-Cl bond as bromine is heavier than chlorine fig.11.



2. The techniques of infrared spectroscopy

We have two types of IR spectrophotometers: The classical and the Fourier Transform spectrophotometers with the interferometer

2.1 The classical IR spectrometers [2, 3]

The main elements of the standard IR classical instrumentation consist of 4 parts (see Fig.12)

1. A light source of irradiation

2. A dispersing element, diffraction grating or a prism

3. A detector

4. Optical system of mirrors

Schematics of a two-beam absorption spectrometer are shown in. Fig. 12.

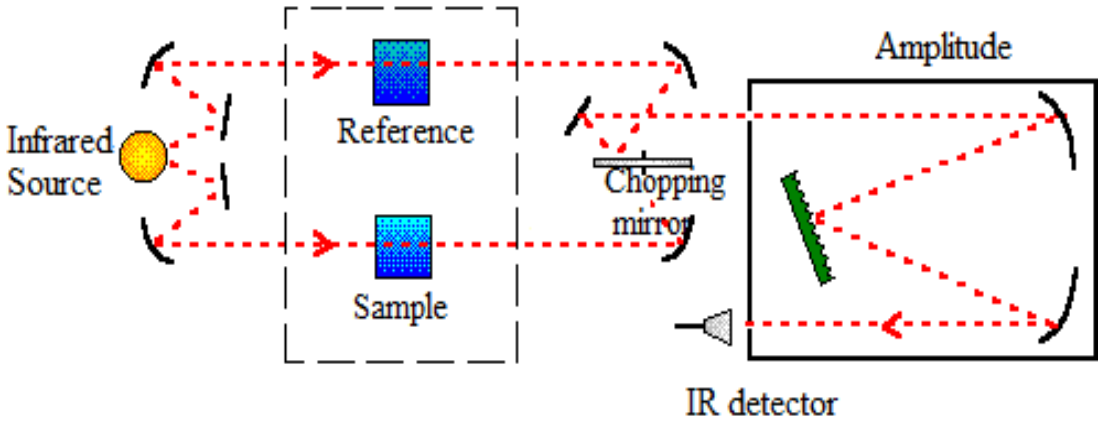


Fig.12. A schematic diagram of the classical dispersive IR spectrophotometer.

The infrared radiation from the source by reflecting to a flat mirror passes through the sample and reference monochromator then through the sample. The beams are reflected on a rotating mirror, which alternates passing the sample and reference beams to the dispersing element and

finally to detector to give the spectrum (see Fig 12). As the beams alternate the mirror rotates slowly and different frequencies of infrared radiation pass to detector.

2.2 Fourier Transform IR spectrometers

The modern spectrometers [8] came with the development of the high performance Fourier Transform Infrared Spectroscopy (FT-IR) with the application of a Michelson Interferometer [9]. Both IR spectrometers classical and modern give the same information the main difference is the use of Michelson interferometer, which allows all the frequencies to reach the detector at once and not one at the time.

The addition, of the lasers to the Michelson interferometer provided an accurate method (see Figs. 13A & 13B) of monitoring displacements of a moving mirror in the interferometer with a high performance computer, which allowed the complex interferogram to be analyzed and to be converted *via* Fourier transform to give spectra.

Fig. 13A. Michelson FT-IR Spectrometer has the following main parts:

1. Light source

2. Beam splitter (half silvered mirror)

3. Translating mirror

4. Detector

5. Optical System (fixed mirror)

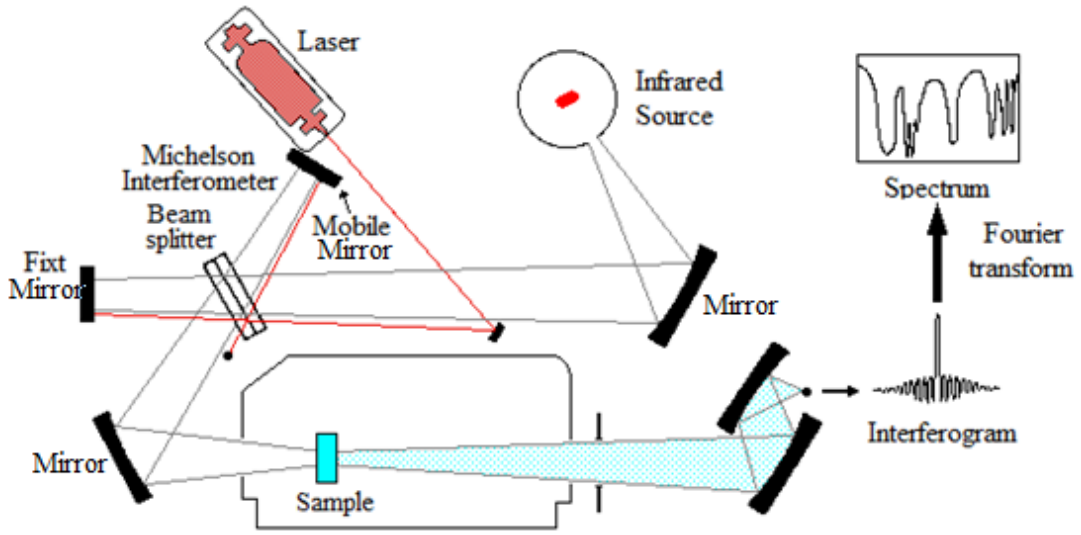


Fig. 13B. Schematic illustration of a modern FTIR Spectrophotometer.

Infrared spectroscopy underwent tremendous advances after the second world war and after 1950 with improvements in instrumentation and electronics, which put the technique at the center of chemical research and later in the 80's in the biosciences in general with new sample handling techniques, the attenuated total reflection method (ATR) and of course the interferometer [10]. The Fourier Transform. IR spectrophotometry is now widely used in both research and industry as a routine method and as a reliable technique for quality control, molecular structure determination and kinetics [11-12] in biosciences(see Fig.14). Here the spectrum of a very complex matter , such as an atheromatic plaque is given and interpreted.

In practice today modern techniques are used and these are the FT-methods. The non- FT methods are the classical IR techniques of dispersion of light with a prism or a diffraction grading. The FT-technique determines the absorption spectra more precisely. A Michelson interferometer should be used today to obtain the IR spectra [13]. The advantage of Ftmethod is that it detects a broad band of radiation all the time (the multiplex or Fellget advantage) and the greater proportion of the source radiation passes through the instrument because of the circular aperture (Jacquinot advantage) rather than the narrow slit used for prisms or diffraction gratings in the classical instrument.

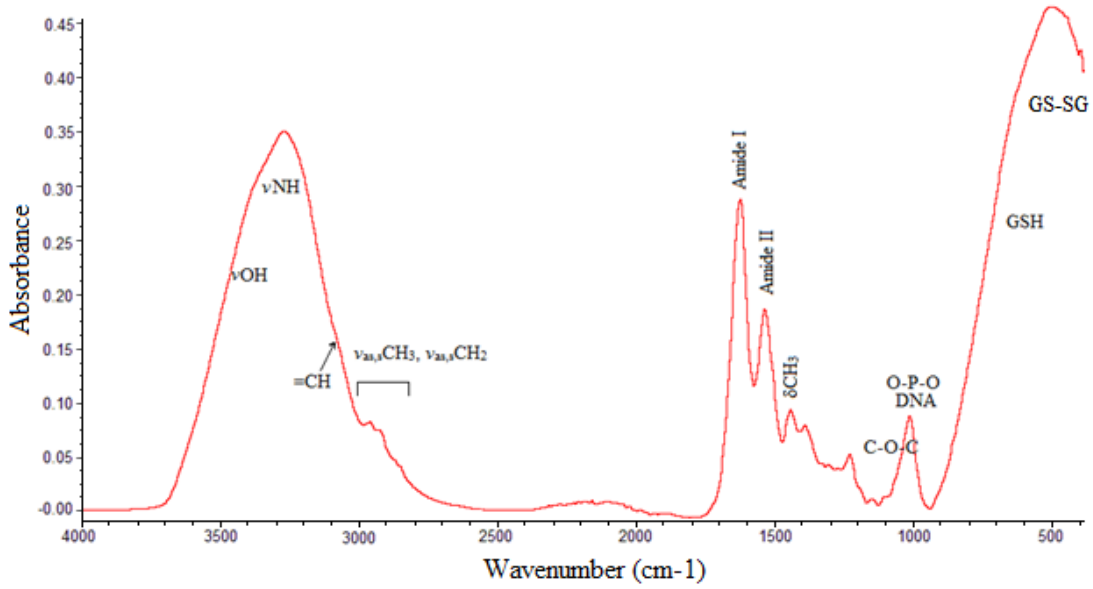


Fig. 14. FT-IR spectrum of a coronary atheromatic plaque is shown with the characteristic absorption bands of proteins, amide bands, O-P-O of DNA or phospholipids, disulfide groups, etc.

2.3 Micro-FT-IR spectrometers The addition of a reflecting microscope to the IR spectrometer permits to obtain IR spectra of small molecules, crystals and tissues cells, thus we can apply the IR spectroscopy to biological systems, such as connective tissues, blood samples and bones,

in pathology in medicine [14, 15-16]. In Fig. 15 is shown the microscope imaging of cancerous breast tissues and its spectrum.

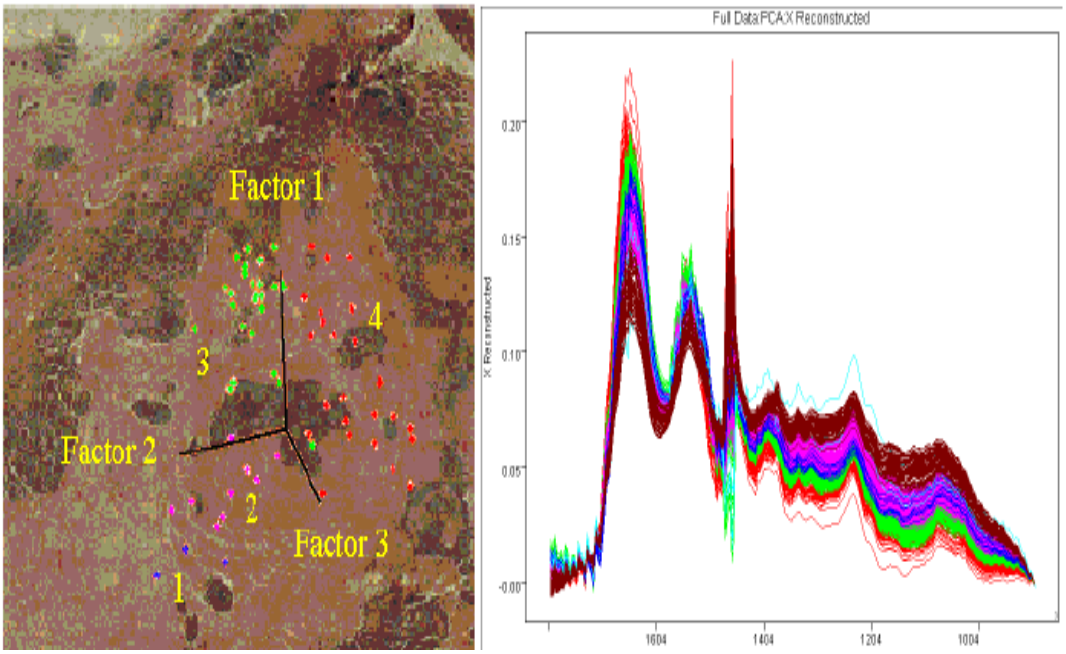


Fig.15. Breast tissue: a 3-axis diagram and the mean spectral components are shown [17].

3. Applications

Infrared spectroscopy is used in chemistry and industry for identification and characterization of molecules. Since an IR spectrum is the "fingerprint" of each molecule IR is used to characterize substances [12, 13]. Infrared spectroscopy is a non destructive method and as such it is useful to study the secondary structure of more complicated systems such as biological molecules proteins, DNA and membranes. In the last decade infrared spectroscopy started to be used to characterize healthy and non healthy human tissues in medical sciences.

IR spectroscopy is used in both research and industry for measurement and quality control. The instruments are now small and portable to be transported, even for use in field trials. Samples in solution can also be

measured accurately. The spectra of substances can be compared with a store of thousands of reference spectra [18]. Some samples of specific applications of IR spectroscopy are the following:

IR spectroscopy has been highly successful in measuring the degree of polymerization in polymer manufacture [18]. IR spectroscopy is useful for identifying and characterizing substances and confirming their identity since the IR spectrum is the "fingerprint" of a substance. Therefore, IR also has a forensic purpose and IR spectroscopy is used to analyze substances, such as, alcohol, drugs, fibers, blood and paints [19-20].

FT-IR was used to simultaneously determine the relative content of major intracellular compositions in different microalgal strains and to explicitly depict the carbon allocation of microalgae under different cultivation conditions. The traditional methods had validated the FT-IR results. FT-IR can be applied in microalgal strain screening, and studying of the dynamics of lipid, carbohydrate, and protein during microalgal cultivation, as well as evaluation of physiological status of microalgae in response to nutrient stress in microalgal metabolism study [21].

Infrared spectroscopy in pharmacy

Infrared IR. spectroscopy has been presented as applied to pharmacy in solution of various specific problems(drug identity, test purity, drug crystalline structures, interactions between active medicaments and excipients, patent of antibiotics, etc). The indisputable importance of IR spectroscopy in the field of (pharmacy sciences, analysis and industry. was proved by its recent successful applications as obligatory/guiding analytical method as well as in combination with other methods(22).

IR spectroscopy and IR techniques have well known advantages in comparison with other analytical methods. The reviews giving information about IR spectroscopy and its applications in pharmacy are presented by Moll (23,24), McDonald (25,26), Danielsson (27), Gonzales (28), Kendall (29), etc.

All information summarized in literatures about IR spectroscopy applications in pharmacy is presented schematically in Fig. 15.

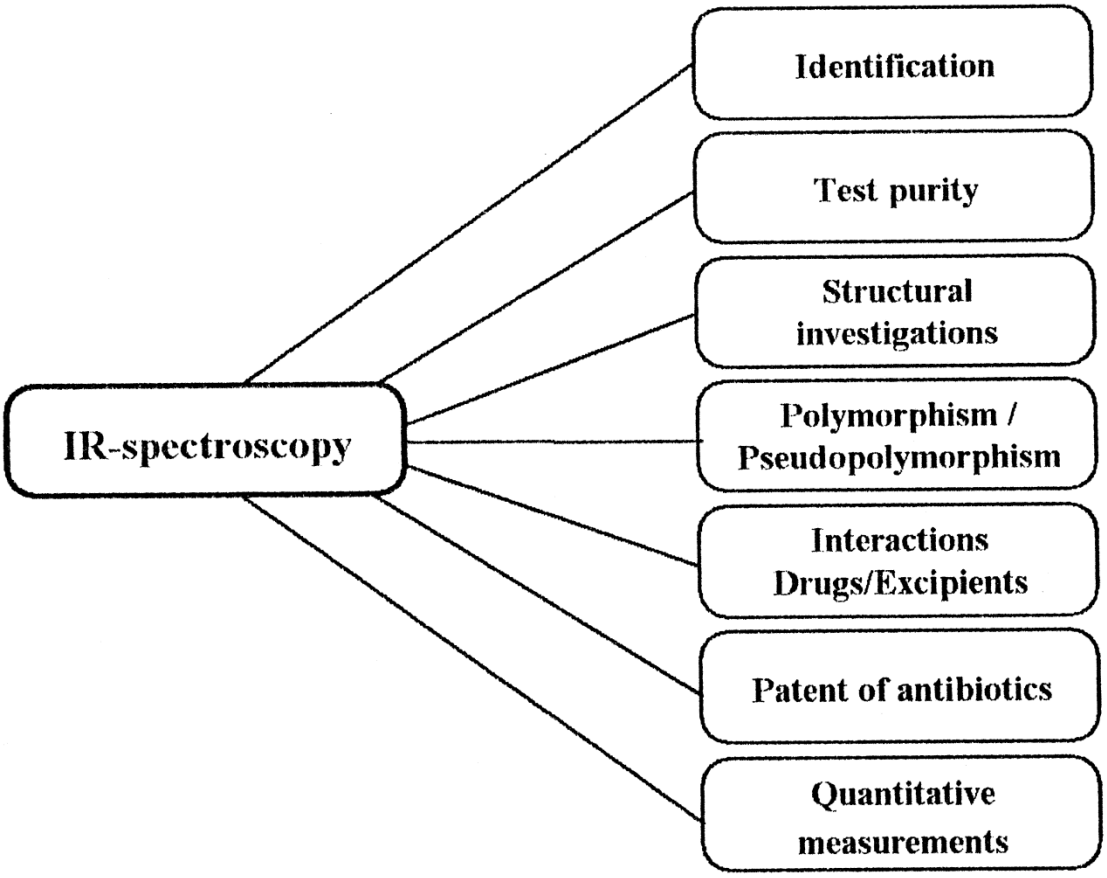


Fig. 15. Schematic presentation of IR spectroscopy in pharmacy.

Drug test purity

IR spectroscopy secures simultaneously verification of drug identity and examination of drug test purity. Usually, the drug identification precedes the drug test purity.

The drugs are the finest products of the chemicopharmaceutical manufacture _industry. Nevertheless, they can contain minimal amounts of pollutants, intermediate and/or disruptive products (each one of them can cause unwanted alteration in drug therapeutic activity). Their discovery is an obligation of the IR spectroscopist–analysts as their removal is a care of the producer. For example: The appearance of the band at 1745 cmy1 in IR spectrum of the Chloramphenicol was shown as a pollution with dichlor acetic acid. This peak was defined as analytical band of the pollution [23,24].

Drugs structural investigations

The deciphering of the structural formula of antibiotic penicillin was made by means of IR spectroscopy _during the Second World War according to the English–American program. [22,23]. Three possible structural formulae have been proposed on the basis of the investigations of large groups of chemists. The study of IR spectrum of the crystal penicillin proved the presence of a strong band at 1780 cm^{-1} related to CO group and the position is corresponding to the structure of b-lactam with condensed cycles. Special synthesized more simple compounds with analogical structures having an absorption band at the same frequency 1780 cm^{-1}, proved the rightness of the choice of the formula. Also, the structure of the antibiotic mycomycin and of its isomers, were established using their IR spectra.

IR spectroscopy in patent of antibiotics

It is well known that the antibiotic's IR spectra are complicated because of the many absorption bands. The overlapping of the bands results in the appearance of a characteristic curve. Although the antibiotic's IR

spectrum is often not interpreted the shape of its common IR spectral curve was considered as characteristic of each antibiotic, too. For example: tetracycline, oxytetracycline, etc). The interpretation in these cases is difficult or nearly impossible, even for the most experienced spectroscopist.

In spite of this, IR spectra were introduced as the only suitable analytical method in patent of the antibiotics. Kendall (28) has given an important information in this matter (a standard presentation of the IR spectral data according to the requirements of the British Chemical Society and those of the Subcommittee on Standard Data-USA).

Finally, IR spectroscopy has also an important role in the field of the patent of the antibiotics.

The connection of IR spectroscopy with pharmaceutical and other sciences

The connection of IR spectroscopy with pharmaceutical and other sciences (Pharmaceutical analysis, Pharmaceutical technology, Pharmaceutical chemistry, Pharmacognosy, Toxicological chemistry and Pharmacology) has been presented in a schema (Fig.15).

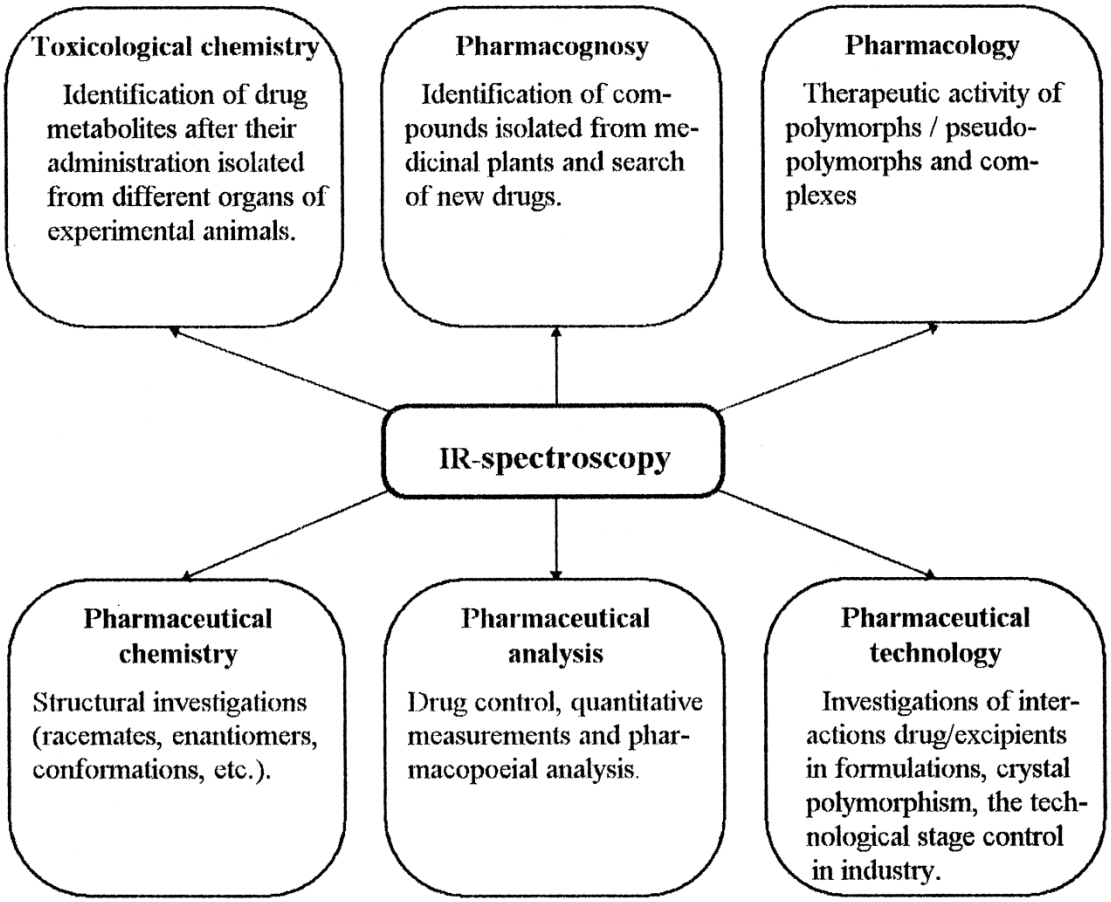


Fig.15. Scheme showing the connection of IR spectroscopy with pharmaceutical and other sciences.

Conclusion

General Uses

•Identification of all types of organic and many types of inorganic compounds

•Determination of functional groups in organic materials

•Determination of the molecular composition of surfaces

•Identification of chromatographic effluents

•Quantitative determination of compounds in mixtures

•Nondestructive method

•Determination of molecular conformation (structural isomers) and stereochemistry (geometrical isomers)

•Determination of molecular orientation (polymers and solutions)

Common Applications

•Identification of compounds by matching spectrum of unknown compound with reference spectrum (fingerprinting)

•Identification of functional groups in unknown substances

The above described IR spectroscopy applications have been presented as proofs for its importance in pharmacy (pharmacy is used in terms of pharmaceutical sciences, pharmaceutical analysis and pharmaceutical industry). The great possibilities of IR spectroscopy have been estimated in successful solutions of various specific pharmaceutical problems (drug identity, drug crystalline structures, interactions between active medicaments and excipients, patents of antibiotics, etc).

The IR spectra provide valuable information on both molecular and submolecular structure of drugs. The alternations of drug molecular structures lead to small IR spectral differences (which is not unexpected). The latter require more attention in order to be detected and proved.

IR spectroscopy has been shown as obligatory/guiding analytical method as well as in combination with other methods. The connection of IR spectroscopy with pharmaceutical and other sciences (Pharmaceutical analysis, Pharmaceutical technology, Pharmaceutical chemistry, Pharmacognosy, Toxicological chemistry and Pharmacology) has been presented in a schema.

Three basic factors are determining IR spectroscopy's increased role and its importance in pharmacy in the future—the pharmaceutical industry growth, the care of high quality drugs and the continuous progress in IR technique-developments.

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